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(54) **VIRAL PARTICLE RELEASED AFTER INFECTION OF MAMMALIAN CELLS BY HUMAN CYTOMEGALOVIRUS (HCMV) CONTAINING A FUSION PROTEIN AND USE THEREOF**

NACH DER INFEKTION VON SÄUGETIERZELLEN MIT EINEM HUMANEN,
FUSIONSPROTEINHALTIGEN ZYTOMEGALIEVIRUS (HCMV) FREIGESETZTER VIRUSPARTIKEL
UND VERWENDUNG DAVON

PARTICULE VIRALE LIBÉRÉE APRÈS INFECTION DE CELLULES DE MAMMIFÈRE PAR UN
CYTOMÉGALOVIRUS HUMAIN (HCMV) CONTENANT UNE PROTÉINE DE FUSION ET
UTILISATION ASSOCIÉE

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(56) References cited:
WO-A1-2006/004661 US-A1- 2004 152 182

- **MERSSEMAN VERONIQUE ET AL: "Exogenous introduction of an immunodominant peptide from the non-structural IE1 protein of human cytomegalovirus into the MHC class I presentation pathway by recombinant dense bodies", JOURNAL OF GENERAL VIROLOGY, SOCIETY FOR GENERAL MICROBIOLOGY, SPENCERS WOOD, GB, vol. 89, no. Part 2, 1 February 2008 (2008-02-01), pages 369-379, XP002494811, ISSN: 0022-1317, DOI: DOI:10.1099/VIR.0.83380-0 cited in the application**
- **VERONIQUE MERSSEMAN ET AL: "Refinement of strategies for the development of a human cytomegalovirus dense body vaccine", MEDICAL MICROBIOLOGY AND IMMUNOLOGY, SPRINGER, BERLIN, DE, vol. 197, no. 2, 5 March 2008 (2008-03-05), pages 97-107, XP019630565, ISSN: 1432-1831 cited in the application**
- **REAP ET AL: "Development and preclinical evaluation of an alphavirus replicon particle vaccine for cytomegalovirus", VACCINE, ELSEVIER LTD, GB, vol. 25, no. 42, 28 September 2007 (2007-09-28), pages 7441-7449, XP022277623, ISSN: 0264-410X, DOI: DOI:10.1016/J.VACCINE.2007.08.016**

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| <ul style="list-style-type: none">• PEPPERL SANDRA ET AL: "Dense bodies of human cytomegalovirus induce both humoral and cellular immune responses in the absence of viral gene expression", JOURNAL OF VIROLOGY, THE AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 74, no. 13, 1 July 2000 (2000-07-01), pages 6132-6146, XP002494810, ISSN: 0022-538X, DOI: DOI:10.1128/JVI.74.13.6132-6146.2000 | <ul style="list-style-type: none">• BECKE S ET AL: "Optimized recombinant dense bodies of human cytomegalovirus efficiently prime virus specific lymphocytes and neutralizing antibodies without the addition of adjuvant", VACCINE, ELSEVIER LTD, GB, vol. 28, no. 38, 31 August 2010 (2010-08-31), pages 6191-6198, XP027211925, ISSN: 0264-410X [retrieved on 2010-07-23] |
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Description

[0001] The present invention is related to a viral particle released after infection of mammalian cells by human cytomegalovirus (HCMV), wherein

- a) the particle is surrounded by a lipid membrane in which viral glycoproteins are embedded,
- b) the particle contains neither viral DNA nor capsids; and
- c) the particle contains a fusion protein comprising one or more parts of the T-cell antigen pp65 and at least one heterologous peptide;

the viral particle for use in a method for the treatment and/or prevention of a disease and the use of such viral particle or of a plurality of such viral particle.

[0002] Infection with the human cytomegalovirus (HCMV) is a major cause of disease in patients following solid organ or haematopoietic stem cell transplantation [1-9]. Furthermore, transmission of the virus during pregnancy is one of the most frequent causes of lasting sequelae in the newborn in the western hemisphere [10;11]. The development of an HCMV vaccine has thus been identified as a high priority goal [12].

[0003] One aim of such vaccine would be to prevent infection in the mother or, at least, cross-placental transmission. Induction of neutralizing antibodies is considered essential to achieve this. In contrast, protection against reactivation and control of infection in transplant recipients is considered to be afforded by cellular responses, particularly mediated through CD8 T cells [13-15]. Therapeutic vaccination of transplant recipients, addressing the lymphocyte response, possibly combined with adoptive T-cell transfer would be desirable to ameliorate the consequences of viral reactivation of HCMV in the post-transplant period [16].

[0004] Despite promising approaches to develop an HCMV vaccine, there is however still no licensed formulation available [10;17-19]. From numerous studies addressing the immune effectors that afford protection, it appears evident that an ideal vaccine against HCMV should induce both antiviral neutralizing antibodies and T lymphocytes [10;17;18;38]. There is, however, concern if such a universal HCMV vaccine may ever be established. A recent clinical study showed that alphavirus replicons expressing HCMV proteins were well tolerated and induced sustained cellular and humoral responses [19]. Another study employed purified gB as vaccine and provided encouraging results, as significant levels of HCMV neutralizing antibodies could be induced [18]. These vaccines hold promise to be used for the prevention of congenital HCMV infection.

[0005] In previous studies in mice, the so-called dense bodies, which are also referred to herein as DB, proved to be surprisingly immunogenic [20]. As generally understood in the art and as also used herein a dense body is a viral particle released after infection of mammalian cells by human cytomegalovirus (HCMV), wherein

- a) the particle is surrounded by a lipid membrane in which viral glycoproteins are embedded,
- b) the particle contains neither viral DNA nor capsids.

[0006] Preferably such dense body also contains a fusion protein comprising one or more parts of the T-cell antigen pp65 and at least one heterologous peptide. Dense bodies and their preparation are also described in international patent application WO 00/53729.

[0007] In view of their surprising immunogenicity, efforts were made to develop DB as an HCMV vaccine. Recent experiments provided experimental evidence that DB can be modified in their antigenic content [21;22]. This was achieved by expressing a fusion protein consisting of the major DB-component pp65 and a heterologous MHC-class I presented peptide from the viral IE1-protein by a recombinant HCMV. This resulted in the formation of recombinant DB (recDB) in infected human foreskin fibroblasts (HFF). The recDB containing the fusion protein were released from infected HFF [21]. Application of these particles to HLA-A2 transgenic HHD-mice induced a CD8⁺ T lymphocyte response, which became apparent after in vitro peptide stimulation of isolated T cells [22]. However, HCMV specific T cells could not be immediately detected when CD8⁺ T cell fractions were tested directly ex-vivo by Elispot analysis. This indicated that, although in principle suitable for therapeutic application, priming was not as prominent as desirable with these particles. Further to that, the yield of these recDB from infected HFF cultures was low.

[0008] Therefore, the problem underlying the present invention was to provide a viral dense body which is suitable as a vaccine, wherein the viral dense body contains a fusion protein comprising one or more parts of the T-cell antigen pp65 and at least one heterologous peptide and wherein preferably the vaccine is capable of eliciting an immune response to the at least one heterologous peptide.

[0009] A further problem underlying the present invention was to provide a viral dense body, wherein the viral dense body contains a fusion protein comprising one or more parts of the T-cell antigen pp65 and at least one heterologous peptide and wherein preferably the dense body is capable of inducing the formation of neutralizing antibodies and/or a CD8⁺ T lymphocyte response.

[0010] A still further problem underlying the present invention was to provide a viral dense body, wherein the viral dense body contains a fusion protein comprising one or more part of the T-cell antigen pp65 and at least one heterologous peptide and wherein the dense body can be prepared at high yields.

[0011] These and other problems are solved by the subject matter of the attached independent claims. Preferred embodiments may be taken from the also attached dependent claims.

[0012] More specifically, the problem underlying the present invention is solved in a first aspect which is also the first embodiment of the first aspect, by a viral particle released after infection of mammalian cells by human cytomegalovirus (HCMV), wherein the viral particle is a dense body, wherein

- a) the particle is surrounded by a lipid membrane in which viral glycoproteins are embedded,
- b) the particle contains neither viral DNA nor capsids; and
- c) the particle contains a fusion protein comprising one or more parts of the T-cell antigen pp65 and at least one antigenic heterologous peptide,

and wherein the at least one heterologous peptide is inserted at amino acid position W175 or A534 of the amino acid sequence of the T-cell antigen pp65 of SEQ ID NO: 1.

[0013] In a second embodiment of the first aspect which is also an embodiment of the first embodiment of the first aspect, the at least one antigenic heterologous peptide is inserted at amino acid position W175 of the amino acid sequence of the T-cell antigen pp65.

[0014] In a third embodiment of the first aspect which is also an embodiment of the first and the second embodiment of the first aspect, the amino acid sequence of the T-cell antigen pp65 comprises an amino acid sequence according to SEQ ID NO: 1.

[0015] In a fourth embodiment of the first aspect which is also an embodiment of the first, the second and the third embodiment of the first aspect, the particle is highly antigenic.

[0016] In a fifth embodiment of the first aspect which is also an embodiment of the first, the second, the third and the fourth embodiment of the first aspect, the particle is capable of inducing the formation of neutralizing antibodies.

[0017] In a sixth embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth and the fifth embodiment of the first aspect, the particle is capable of inducing a CD8⁺ T lymphocyte response.

[0018] In a seventh embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth and the sixth embodiment of the first aspect, the at least one antigenic heterologous peptide is a MHC-class I presented antigen.

[0019] In an eighth embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth and the seventh embodiment of the first aspect, the at least one antigenic heterologous peptide comprises or is formed of one or more parts of one or more proteins which is/are different from pp65.

[0020] In a ninth embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh and the eighth embodiment of the first aspect, the at least one antigenic heterologous peptide comprises or is one or more parts of an HCMV glycoprotein.

[0021] In a tenth embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh, the eighth and the ninth embodiment of the first aspect, the at least one antigenic heterologous peptide comprises or is one or more parts of the HCMV glycoprotein gB.

[0022] In an eleventh embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh, the eighth and the ninth embodiment of the first aspect, the at least one antigenic heterologous peptide comprises or is one or more parts of the HCMV glycoprotein gH.

[0023] In a twelfth embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh, the eighth and the ninth embodiment of the first aspect, the at least one antigenic heterologous peptide comprises or consists of at least two HCMV glycoproteins which are variants of a particular glycoprotein from different HCMV strains.

[0024] In a thirteenth embodiment of the first aspect which is also an embodiment of the twelfth embodiment of the first aspect, one of the at least two variants of the particular glycoprotein is the variant of the HCMV Towne strain and the other of the at least two variants of the particular glycoprotein is the variant of the HCMV Ad 169 strain.

[0025] In a fourteenth embodiment of the first aspect which is also an embodiment of the twelfth and the thirteenth embodiment of the first aspect, the glycoprotein is the gB protein of HCMV.

[0026] In a fifteenth embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh and the eighth embodiment of the first aspect, the at least one antigenic heterologous peptide comprises or is one or more parts of the HCMV protein IE1.

[0027] In a sixteenth embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh and the eighth embodiment of the first aspect, the at least one antigenic heterologous peptide comprises or is one or more parts of an HCMV glycoprotein and one or more parts of the HCMV

protein IE1.

[0028] In a seventeenth embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh and the eighth embodiment of the first aspect, the at least one antigenic heterologous peptide is one or more parts of a protein which is part of a human pathogen other than HCMV.

[0029] In an eighteenth embodiment of the first aspect which is also an embodiment of the seventeenth embodiment of the first aspect, the protein which is part of a human pathogen other than HCMV, is a protein against which cytotoxic T lymphocytes are formed in humans upon natural infection of humans with the human pathogen other than HCMV.

[0030] In a nineteenth embodiment of the first aspect which is also an embodiment of the eighteenth embodiment of the first aspect, the human pathogen other than HCMV is human pathogen selected from the group comprising HIV-1, HBV, HCV and influenza.

[0031] In a twentieth embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh, the eighth, the ninth, the tenth, the eleventh, the twelfth, the thirteenth, the fourteenth, the fifteenth, the sixteenth, the seventeenth, the eighteenth and the nineteenth embodiment of the first aspect, the fusion protein is a fusion protein comprising a full-length T-cell antigen pp65 and at least one antigenic heterologous peptide.

[0032] In a twenty-first embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh, the eighth, the ninth, the tenth, the eleventh, the twelfth, the thirteenth, the fourteenth, the fifteenth, the sixteenth, the seventeenth, the eighteenth, the nineteenth and the twentieth embodiment of the first aspect, the viral particle, or a plurality of such a particle, is for the manufacture of a medicament for the treatment and/or prevention of a disease.

[0033] In a twenty-second embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh, the eighth, the ninth, the tenth, the eleventh, the twelfth, the thirteenth, the fourteenth, the fifteenth, the sixteenth, the seventeenth, the eighteenth, the nineteenth and the twentieth embodiment of the first aspect, the viral particle, or a plurality of such a particle, is for use in a method for the treatment and/or prevention of a disease.

[0034] In a twenty-third embodiment of the first aspect which is also an embodiment of the twenty-first and the twenty-second embodiment of the first aspect, the disease is a disease which can be treated and/or prevented by the formation of neutralizing antibodies against the at least one antigenic heterologous peptide or a derivative thereof, or by the induction of a CD8⁺ T lymphocyte response against the at least one antigenic heterologous peptide or a derivative thereof.

[0035] In a twenty-fourth embodiment of the first aspect which is also an embodiment of the first, the second, the third, the fourth, the fifth, the sixth, the seventh, the eighth, the ninth, the tenth, the twelfth, the thirteenth, the fourteenth, the fifteenth, the sixteenth, the seventeenth, the eighteenth, the nineteenth and the twentieth embodiment of the first aspect, the viral particle, or a plurality of such a particle, is for the manufacture of a vaccine.

[0036] In a twenty-fifth embodiment of the first aspect which is also an embodiment of the twenty-fourth embodiment of the first aspect, the vaccine is for the treatment and/or prevention of HCMV infection.

[0037] In a twenty-sixth embodiment of the first aspect which is also an embodiment of the twenty-fourth embodiment of the first aspect, the vaccine is for the treatment and/or prevention of side effects of transplantation.

[0038] In a twenty-seventh embodiment of the first aspect which is also an embodiment of the twenty-sixth embodiment of the first aspect, the transplantation is transplantation of a solid organ or haematopoietic stem cells.

[0039] In a twenty-eighth embodiment of the first aspect which is also an embodiment of the twenty-sixth and the twenty-seventh embodiment of the first aspect, the side effect is caused by or is going along with a HCMV infection.

[0040] The problem underlying the present invention is solved in a second aspect which is also the first embodiment of the second aspect, by use of a viral particle according to any one of the first, the second, the third, the fourth, the fifth, the sixth, the seventh, the eighth, the ninth, the tenth, the eleventh, the twelfth, the thirteenth, the fourteenth, the fifteenth, the sixteenth, the seventeenth, the eighteenth, the nineteenth and the twentieth embodiment of the first aspect for the manufacture of a medicament for the treatment and/or prevention of a diseases or side effects, whereby the disease or side effects is a disease or are side effects as defined in each and any of the preceding embodiments and of each and any aspect.

[0041] The problem underlying the present invention is solved in a third aspect which is also the first embodiment of the third aspect, by use of a viral particle according to any one of the first, the second, the third, the fourth, the fifth, the sixth, the seventh, the eighth, the ninth, the tenth, the eleventh, the twelfth, the thirteenth, the fourteenth, the fifteenth, the sixteenth, the seventeenth, the eighteenth, the nineteenth and the twentieth embodiment of the first aspect for the manufacture of a vaccine for the treatment and/or prevention of a disease or side effects, whereby the disease or side effects is a disease or are side effects as defined in each and any of the preceding embodiments and of each and any aspect.

[0042] Based on the insight that the insertion site for heterologous peptide sequences into pp65 was critical for the efficiency of subsequent DB formation, the present inventors have surprisingly found that the fusion protein which is contained in the viral particles of the invention and which comprises one or more parts of the T-cell antigen pp65 and

at least one heterologous peptide, confers advantageous characteristics to the DB if the heterologous peptide is inserted at amino acid position W 175 or at amino acid position A534 of the pp65 amino acid sequence. The advantageous characteristics of these fusion proteins comprise the induction of neutralizing antibodies against the heterologous peptide, the induction of a CD8⁺ T lymphocyte response to the heterologous peptide and the production of such DB at a high yield. Furthermore, these two loci in pp65 allowed efficient formation and release of DB containing said fusion proteins. The CD8⁺ T lymphocyte response observed for these two fusion proteins primed CD8 T cell responses, which are readily detectable by ex vivo Elispot analyses of CD8 T cell fractions.

[0043] The T-cell antigen pp65, which is also referred to in the art as UL 83, has been described by Pande, H. et al. (Pande, H, Lee, T.D., Churchill, M.A. and Zaia, J.A, "Structural analysis of a 64-kDa major structural protein of human cytomegalovirus (Towne): identification of a phosphorylation site and comparison to pp65 of HCMV (AD169); Virology 178 (1), 6-14 (1990)).

[0044] Little is known about the function of pp65 and about domains, important for function and proper folding of the protein. Consequently, there is no rationale for insertion site selection that would definitely spare functionally relevant regions within pp65. Insertion site selection was performed in a way to avoid regions conserved in beta-herpesviruses and also predicted secondary structures (α -helices and β -sheet). This was done assuming that conserved regions, α -helices and β -sheets could be structurally and functionally important and should not be destroyed. However, most of these mutants failed to efficiently form recDB, indicating that this strategy could not identify proper insertion sites. In the light thereof the present inventors surprisingly found that the insertion sites in RV-SB3 and RV-SB6 proved to be more appropriate for recDB formation.

[0045] As is shown in the present application exogenous introduction of chimeric pp65 by DB-SB3 and DB-SB6 into HFF led to efficient presentation of pp65NLV and IE1TMY. Application of these particles to HLA-A2 transgenic HHD mice primed a CD8 T cell response against both peptides that could be detected after in-vitro expansion of these T cells with the cognate peptide. Similar results have also been obtained in previous experiments, using recDB with insertion of the IE1-peptide at position 548 of pp65 [21;22]. However, using the latter recDB, CD8 T cells against either peptide were undetectable directly ex vivo, suggesting that priming was inefficient. Application of DB-SB3, in contrast, led to readily detectable frequencies of pp65NLV-specific and IE1TMY-specific CD8 T cells. This indicates that these recDB were highly immunogenic and induced T cells against both the endogenous and the heterologous peptide.

[0046] In an embodiment of the invention the heterologous peptide is an antigenic peptide and more preferably an antigenic heterologous peptide.

[0047] In an embodiment of the present invention that the heterologous peptide is different from the IE1 protein of HCMV or a part thereof.

[0048] In an embodiment of the present invention the length of a peptide is from about 4 to 40 amino acid residues, preferably about 6 to 25 amino acid residues and more preferably about 8 to 15 amino acid residues and most preferably about 8-12 amino acid residues.

[0049] As preferably used, any wording which specifies the limits of a range such as, e. g., "from 1 to 5" means any integer from 1 to 5, i. e. 1, 2, 3, 4 and 5. In other words, any range that is defined by two integers comprises both the two integers defining said limits of the definition and any integer comprised by or contained within said range.

[0050] In an embodiment of the present invention the antigenicity of the viral particle of the invention is increased compared to the dense bodies of the prior art, more specifically those dense bodies where the dense body contains a fusion protein comprising the full-length T-cell antigen pp65.

[0051] In an embodiment of the present invention the formation of the viral particle of the invention is increased compared to the formation of dense bodies as described in [25].

[0052] It will be acknowledged by a person skilled in the art that, preferably, the HCMV protein IE1 is also referred to as ppUL123.

[0053] It will also be acknowledged that the fusion protein contained in the particle is, in a preferred embodiment a chimeric protein.

[0054] The present invention is now further illustrated by reference to the following figures and examples from which further advantages, features, and embodiments may be taken, wherein

Fig. 1 shows the result of an indirect immuno fluorescence analysis of HFF, infected with HCMV recombinants;

Fig. 2 shows the result of an immuno blot analysis (A), the result of a quantitative DNA-PCR analysis as a diagram indicating genome copies/ml as a function of time (d.p.i) for various fusion proteins (B), and the result of a further immuno blot analysis (C);

Fig. 3 shows the result of an IFN- γ -Elispot analysis as a diagram indicating the percentage of responding CD8⁺ cells for various fusion proteins either using either pp65NLV-CTL (A) or IE1TMY-CTL (B) as responder cells;

Fig. 4 shows the result of an IFN- γ -Elispot analysis as a diagram indicating the percentage of responding CD8⁺ cells for various fusion proteins either using RMA-S cells as stimulator cells; and

Fig. 5 shows the amino acid sequence of full-length pp65 (SEQ ID NO: 1)

[0055] Fig. 1 shows the result of an indirect immuno fluorescence analysis of HFF, infected with HCMV recombinants. Cells were infected with the indicated viruses for four days and were subsequently processed for immuno fluorescence analysis. The configuration of the recombinant pp65, expressed by each virus is indicated below the micrographs. The N-terminal amino acid of pp65, flanking the insertion site is denoted.

[0056] Fig. 2 shows the expression of pp65-fusion proteins, replication of recombinant viruses and efficiency of packaging of fusion proteins into virions and DB. (A), Immuno blot analysis of 2- and 4-day infected HFF. Cells were infected at an m.o.i. of 2 and were collected at the indicated times for analysis. Filters were probed with pp65-specific monoclonal antibody 65-33. Amounts of proteins in each lane were normalized against β -actin. (B), Quantitative DNA-PCR analysis of viral genomes in the cell culture supernatant of infected HFF. Cells were infected at an m.o.i. of 10 genomes/cell. Culture supernatants were collected at the indicated time points and frozen. PCR analysis was performed in parallel in the same assay. (C), Immunoblot analysis of pp65, packaged in recDB or virions. Cells were infected with the recombinant viruses or with wt RV-HB5 for 6-7 days. Supernatants were subjected to glycerol-tartrate gradient centrifugation to collect virions- and DB-fractions. These fractions were analyzed by immunoblot, using monoclonal antibody 65-33 or, as internal standard, using a monoclonal antibody against viral gB.

[0057] Fig. 3 shows the result of an IFN- γ -Elispot analysis of MHC-class I presentation by HLA-A2 positive HFF, treated with recDB. Cells were treated with the indicated DB and were subsequently used as stimulator cells in IFN- γ -Elispot analysis, using either pp65NLV-CTL (A) or IE1TMY-CTL (B) as responder cells.

[0058] Fig. 4 shows the result of an ex vivo IFN- γ -Elispot analysis of CD8⁺ enriched spleen cells from HHD-mice, immunized with recDB. RMA-S cells, loaded with the cognate peptides were taken as stimulator cells. Bar sizes represent most probable frequencies of IFN- γ secreting CD8 T cells, as determined by linear regression analysis, described by Böhm et al [37]. Error bars indicate 95% confidence intervals.

Example 1: Materials and Methods

1. Cells

[0059] Primary human foreskin fibroblasts, CTL lines and T2 cells were cultured as described before [23]. RMA-S cells [24] were grown in RPMI 1640 medium (PAA Laboratories, Cölbe, Germany) supplemented with 10% FCS, 2 mM L-glutamine, 50 mg gentamicin L-1 and 5 μ M β -mercaptoethanol.

2. Plasmids and viruses
For mutagenesis of the viral DNA, the HCMV bacterial artificial chromosome (BAC) pHB5 [25] was used. Mutagenesis of pHB5 was performed according to the galK positive/negative selection procedure [26] as described elsewhere [21]. The DNA sequence inserted into the UL83 open reading frame encoded the HLA-A2 presented peptide IE1TMY [IE1297-305], flanked by additional amino acids to enable accurate proteasomal processing. The additional polypeptide fused to pp65 reads TSDACMMTMYGGISLLSEFC, with the HLA-A2 presented nonapeptide being underlined. Viral reconstitution from BAC clones was performed according to Hobom *et al.* [27].

[0060] Virus stocks were generated, titrated and quantified either by counting IE1 positive cells at 48 hours p.i. or by TaqMan DNA PCR analysis of extracellular viral genomes as described [28]. HFF were infected for 7 days. Infection at a moi of 0.1 resulted in a number of roughly 10 intracellular viral genomes.

3. Dense body purification, indirect immunofluorescence analysis and immunoblotting

[0061] DB were purified from late-stage infected HFF by glycerol-tartrate gradient ultracentrifugation as originally published by Irmieri and Gibson [29] and described previously [20]. Indirect immunofluorescence analysis was carried out as described [30]. The pp65 was labelled by using monoclonal antibody 65-33 (kindly provided by W. Britt, University of Alabama, Birmingham, Alabama, USA) and FITCconjugated secondary antibodies (DAKO, Hamburg, Germany). The nucleus was counterstained with DAPI. Data from immunofluorescence analyses were collected using an Axiophot-1 microscope (Zeiss) at magnification of 1000fold. For immunoblotting, protein samples were denatured under reducing conditions, separated by SDS-PAGE and transferred onto nitrocellulose membranes (Millipore, Schwalbach, Germany) by electroblotting at 400 mA for 1 h 45 min. The membranes were incubated with antibodies against pp65, β -actin (Rockland, Gilbertsville, PA, USA) and glycoprotein B (gB) [31]. Western blots were probed with anti-mouse or anti-rabbit secondary antibodies, conjugated to ALEXA Fluor 680 (Invitrogen, Karlsruhe, Germany) or IRdye 800 (Rockland). Blotted proteins were detected and quantified using the Odyssey infrared imaging system (LI-COR, Lincoln, Nebraska, USA).

2.4 Interferon γ - Elispot assay of HFF incubated with recDB

[0062] Enzyme linked immunospot (Elispot) assays were performed as described before [23;32]. CTL lines specific for the HLA-A0201 (A2) restricted HCMV-derived peptides pp65495-503 (pp65NLV-CTL) [33;34] and IE1297-305 (IE1TMY-CTL) [35] were used in these analyses. The CTL lines had been generated by immunizing HLA-A2/huCD8 double-transgenic (tg) mice [23].

2.5 HLA-A2 transgenic mouse model

[0063] 8-12 week-old HLA-A2 transgenic mice (HHD mice, [36]) were immunized intraperitoneally with 6 μ g DB of RV-HB5 (DB-HB5), RV-SB3 (DB-SB3) or RV-SB6 (DB-SB6), respectively, or with PBS. Lymphocytes were prepared from the spleens at day 7 after immunization. CD8 T cells were enriched by MACS sorting and the frequency of IFN- γ secreting cells was analysed by Elispot directly *ex vivo*, using peptide loaded RMA-S HHD or T2 stimulator cells. In this setting the Elispot plates from Millipore (Schwalbach/Ts., Germany) were used. The Elispot assay was performed according to the manufacturer's recommendations. Frequencies of responding cells were determined by linear regression analysis as described by Böhm et al [37].

Example 2: Insertion site selection is critical for formation of cytoplasmic DB

[0064] As will be illustrated in this example, selection of the insertion site of the heterologous peptide is critical for formation of cytoplasmic DB.

[0065] The potential of a vaccine candidate to be further developed critically depends on the yield that can be achieved for further up-scaling. In previous work, proof could be provided that DB can be modified in their antigenic content by fusing a heterologous peptide sequence to the tegument protein pp65 [21]. However, cells infected with the respective recombinant of HCMV released only limited amounts of recDB. Therefore the hypothesis was tested that insertion of heterologous sequence such as the amino acid sequence of a heterologous peptide at other sites within pp65 would enhance the yield of recDB.

[0066] Five sites in different portions of the molecule were selected for insertion (Fig. 1). The inserted peptide consisted of 20 amino acids encompassing the HLA-A2 presented nonapeptide TMYGGISLL (IE1TMY) from the HCMV IE1 protein [35]. Recombinant viruses were generated by modifying the HCMV BAC plasmid pHB5 [25], using the galk-based selection procedure [21;22]. Recombinant BAC-plasmids were analyzed for accuracy by restriction endonuclease digestion and nucleotide sequencing of the insertion site (data not shown). Reconstitution of recombinant viruses was subsequently performed by transfection of the BAC-plasmids into HFF. The resulting viruses were tested for DB formation. One hallmark of HCMV infection of HFF is the cytoplasmic accumulation of DB, which can be visualized by indirect immuno fluorescence analysis (IFA). Consequently, HFF were infected with the newly generated mutants and IFA was performed at 4 days post infection (d.p.i.), using a pp65-specific antibody (Fig.1). Only cells infected with the mutants RV-SB3 and RV-SB6 showed the formation of cytoplasmic DB in a way comparable to the formation of DB in parental-virus infected cells. In contrast, no or only little DB formation was seen in RV-SB2, RV-SB4, and RV-SB5 infected cells. Note that in these cells, the nucleo-cytoplasmic translocation of pp65, which is typically seen in late-stage infected HFF was impaired. These results indicated that site selection for insertion of heterologous peptide sequences within pp65 was critical for recDB formation. Based on the IFA-results the viruses RV-SB3 and RV-SB6 were chosen for further analysis.

Example 3: Comparing RV-SB3 and RV-SB6 with the parental strain

[0067] As will be shown in this example, recombinant viruses RV-SB3 and RV-SB6 are comparable to the parental strain with respect to pp65-expression, virion release and packaging of pp65 into particles.

[0068] Expression levels of pp65 in RV-SB3- and RV-SB6-infected HFF were tested by immunoblot analysis. Cells were infected with either virus or with parental RVHB5 for two or four days, respectively. Cells lysates were subjected to quantitative immunoblot analysis, using the Odyssey infrared imaging system. The amount of cellular actin was taken as internal standard (Fig. 2A). Expression levels of pp65-fusion proteins in RV-SB3- and RV-SB6-infected cells was reduced at 2 days p.i., compared to levels of pp65 in cells infected with parental RV-HB5. However, at 4 days p.i., the levels of the two fusion proteins appeared to be even higher as that of the pp65 in RV-HB5 infected cells. This indicated that sufficient protein was synthesized in RV-SB3- and RV-SB6-infected cells to direct the synthesis of recDB.

[0069] To test the capacity of the recombinant strains to replicate in HFF, cells were infected at an m.o.i. of 0.1, resulting in 10 genome copies/cell and culture supernatant was collected in daily intervals until 7 days p.i.. Viral DNA released into the supernatant was quantified using quantitative PCR analysis (Fig.2B). Both RV-SB3 and RV-SB6 proved to replicate to similar levels as parental RV-HB5.

[0070] Packaging of the fusion proteins was finally analyzed by immunoblot analysis of purified virions and DB, collected from infected HFF supernatants by glycerol gradient centrifugation. In this case, normalization was performed using the viral envelope glycoprotein B (gB) as internal standard. No differences in the packaging of pp65 into virions or DBs were found when the recombinant strains were compared to their parental strain (Fig. 2C). Taken together these results indicated that both RV-SB3 and RVSB6 were comparable to RV-HB5 in expression and packaging of pp65 and in replication in infected cells.

Example 4: Expression of both pp65NLV and IE1TMY by DB-SB3- or DB-SB6-treated HFF

[0071] The present example shows that both pp65NLV and IE1TMY are presented by DB-SB3- or DB-SB6-treated HFF.

[0072] One goal of using recDB for vaccine development would be to support cytotoxic T cell reconstitution in patients following transplantation. These cells have been shown to be of critical importance for the prevention of viral reactivation and disease ([13;15] reviewed in [16]). It thus tested whether the recDB were capable to introduce both the pp65-derived pp65NLV and the IE1-derived IE1TMY into the MHC-class I presentation pathway of HFF.

[0073] Accordingly, cells were treated with recDB and were subsequently subjected to interferon- γ Elispot analysis (Fig. 3). As responder cells, CTL clones against both peptides (pp65NLV-CTL; IE1TMY-CTL) were used [23]. Both DB-SB3- and DB-SB6-treated cells presented IE1TMY and pp65NLV. The pp65NLV-presentation was comparable between recDB and wt-DB, indicating that the insertion of the IE1-derived sequence did not impair pp65-presentation in HFF. Treatment of cells with DB-SB3 led to numbers of responding IE1TMY-CTL to levels that were comparable to the pp65-specific response. However, treatment of cells with DB-SB6 led to a markedly reduced number of positive spots in the IE1-specific assay. This indicated that the ability of HFF to present the IE1-peptide after DB incubation was sensitive to the site, where the peptide was inserted into pp65.

Example 5: Immunization with recombinant DB (recDB) results in IE1TMY-specific and pp65NLV-specific CD8 T cells

[0074] This example shows that immunization with recombinant DB (recDB) primes significant frequencies IE1TMY-specific and pp65NLV-specific CD8 T cells.

[0075] Previous experiments had shown that recDB could induce IE1TMY-specific CD8 T cells in mice. These cells were, however, only detectable after in-vitro stimulation of CD8 T cell fractions from immunized mice with the cognate peptide. No responding CD8 T cells were detected directly ex-vivo, indicating that the total number of specific cells, and thus the overall response to those recDB was low [21]. To evaluate the immunological potential of the newly established recDB, HLA-A2 transgenic HHD-mice were immunized with the different DB in the absence of adjuvant. As expected, responding CD8 T cells specific for IE1TMY or pp65NLV were detectable after in-vitro stimulation (data not shown).

[0076] Cells from immunized mice were also tested directly ex-vivo. For this, the CD8⁺ fractions of spleen cells were separated by MACS-sorting and tested in Elispot analysis, using peptide loaded antigen presenting cells. CD8 T cells, specific for IE1TMY could be detected after immunization with both DB-SB3 and DB-SB6 (Fig. 4). The IE1-specific response primed by DB-SB3 appeared be stronger than the response induced by DB-SB6, but both reached clearly detectable levels.

[0077] After immunization with DB-SB3, CD8 T cells reactive against the pp65NLV could be detected to a roughly threefold level, compared to wt-DB.

[0078] Surprisingly, however, this response was undetectable in the experimental setting chosen after immunization with DB-SB6 and a pp65NLV-specific response could not be detected ex vivo. This result was confirmed in a second experiment and indicated that the CD8 T cell response, primed against the immunodominant pp65NLV-peptide by immunizing with DB-SB6 was inefficient. (Fig. 4). Comparable results were obtained when T2 cells were chosen for antigen presentation (data not shown). Furthermore, this indicates that these cells were induced in only low frequencies below the detection limit of the assay. In accordance therewith, restimulation of pp65NLV-specific T cells in vitro was delayed compared to IE1TMY-specific cells (data not shown).

[0079] Taken together these experiments particularly proved the potential of DB-SB3 to prime a CD8 T cell response against a heterologous antigenic peptide.

List of references:

[0080] In the instant application it is referred to the various references as follows:

[1] Mwintshi K, Brennan DC. Prevention and management of cytomegalovirus infection in solid-organ transplantation. Expert Rev Anti Infect Ther 2007; 5(2):295-304.

[2] Rubin RH. The pathogenesis and clinical management of cytomegalovirus infection in the organ transplant recipient: the end of the 'silo hypothesis'. *Curr Opin Infect Dis* 2007; 20(4):399-407.

[3] Arthurs SK, Eid AJ, Pedersen RA, Dierkhising RA, Kremers WK, Patel R, et al. Delayed-onset primary cytomegalovirus disease after liver transplantation. *Liver Transpl* 2007; 13(12):1703-9.

[4] Arthurs SK, Eid AJ, Pedersen RA, Kremers WK, Cosio FG, Patel R, et al. Delayed-onset primary cytomegalovirus disease and the risk of allograft failure and mortality after kidney transplantation. *Clin Infect Dis* 2008; 46(6):840-6.

[5] Peggs KS. Cytomegalovirus following stem cell transplantation: from pharmacologic to immunologic therapy. *Expert Rev Anti Infect Ther* 2004; 2(4):559-73.

[6] Hebart H, Einsele H. Clinical aspects of CMV infection after stem cell transplantation. *Hum Immunol* 2004; 65(5):432-6.

[7] Ljungman P. Risk assessment in haematopoietic stem cell transplantation: viral status. *Best Pract Res Clin Haematol* 2007; 20(2):209-17.

[8] Boeckh M, Nichols WG, Papanicolaou G, Rubin R, Wingard JR, Zaia J. Cytomegalovirus in hematopoietic stem cell transplant recipients: Current status, known challenges, and future strategies. *Biol Blood Marrow Transplant* 2003; 9(9):543-58.

[9] Boeckh M, Nichols WG. The impact of cytomegalovirus serostatus of donor and recipient before hematopoietic stem cell transplantation in the era of antiviral prophylaxis and preemptive therapy. *Blood* 2004; 103(6):2003-8.

[10] Plotkin SA. Cytomegalovirus vaccines. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*. 5 ed. Elsevier, 2008: p. 1147-54.

[11] Cheeran MC, Lokensgard JR, Schleiss MR. Neuropathogenesis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. *Clin Microbiol Rev* 2009; 22(1):99-126, Table.

[12] Stratton KR, Durch JS, Lawrence R.S. *Vaccines for the 21st Century. A Tool for Decisionmaking*. Washington, DC: National Academy Press, 2001.

[13] Reddehase MJ, Mutter W, Münch K, Bühring HJ, Koszinowski UH. CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity. *J Virol* 1987; 61:3102-8.

[14] Wills MR, Carmichael AJ, Sissons JG. Adaptive Cellular Immunity to Human Cytomegalovirus. In: Reddehase MJ, editor. *Cytomegalovirus: Molecular Biology and Immunology*. Caister Academic Press, Wymondham, Norfolk, U.K., 2006: p. 341-65.

[15] Walter EA, Greenberg PD, Gilbert MJ, Finch RJ, Watanabe KS, Thomas ED, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med* 1995; 333(16):1038-44.

[16] Herr W, Plachter B. Cytomegalovirus and varicella-zoster virus vaccines in hematopoietic stem cell transplantation. *Expert Rev Vaccines* 2009; 8(8):999-1021.

[17] Schleiss MR. Cytomegalovirus vaccine development. *Curr Top Microbiol Immunol* 2008; 325:361-82.

[18] Pass RF, Zhang C, Evans A, Simpson T, Andrews W, Huang ML, et al. Vaccine prevention of maternal cytomegalovirus infection. *N Engl J Med* 2009; 360(12):1191-9.

[19] Bernstein DI, Reap EA, Katen K, Watson A, Smith K, Norberg P, et al. Randomized, double-blind, Phase 1 trial of an alphavirus replicon vaccine for cytomegalovirus in CMV seronegative adult volunteers. *Vaccine* 2009; 28(2):484-93.

[20] Pepperl S, Münster J, Mach M, Harris JR, Plachter B. Dense bodies of human cytomegalovirus induce both

humoral and cellular immune responses in the absence of viral gene expression. *J Virol* 2000; 74(13):6132-46.

[21] Mersseman V, Besold K, Reddehase MJ, Wolfrum U, Strand D, Plachter B, et al. Exogenous introduction of an immunodominant peptide from the nonstructural IE1 protein of human cytomegalovirus into the MHC class I presentation pathway by recombinant dense bodies. *J Gen Virol* 2008; 89(Pt2):369-79.

[22] Mersseman V, Bohm V, Holtappels R, Deegen P, Wolfrum U, Plachter B, et al. Refinement of strategies for the development of a human cytomegalovirus dense body vaccine. *Med Microbiol Immunol* 2008; 197(2):97-107.

[23] Besold K, Frankenberg N, Pepperl-Klindworth S, Kuball J, Theobald M, Hahn G, et al. Processing and MHC class I presentation of human cytomegalovirus pp65-derived peptides persist despite gpUS2-11-mediated immune evasion. *J Gen Virol* 2007; 88(Pt 5):1429-39.

[24] Townsend A, Ohlen C, Bastin J, Ljunggren HG, Foster L, Karre K. Association of class I major histocompatibility heavy and light chains induced by viral peptides. *Nature* 1989; 340(6233):443-8.

[25] Borst EM, Hahn G, Koszinowski UH, Messerle M. Cloning of the human cytomegalovirus (HCMV) genome as an infectious bacterial artificial chromosome in *Escherichia coli*: a new approach for construction of HCMV mutants. *J Virol* 1999; 73(10):8320-9.

[26] Warming S, Costantino N, Court DL, Jenkins NA, Copeland NG. Simple and highly efficient BAC recombineering using galK selection. *Nucleic Acids Res* 2005; 33(4):e36.

[27] Hobom U, Brune W, Messerle M, Hahn G, Koszinowski UH. Fast screening procedures for random transposon libraries of cloned herpesvirus genomes: mutational analysis of human cytomegalovirus envelope glycoprotein genes. *J Virol* 2000; 74(17):7720-9.

[28] Besold K, Wills M, Plachter B. Immune evasion proteins gpUS2 and gpUS11 of human cytomegalovirus incompletely protect infected cells from CD8 T cell recognition. *Virology* 2009; 391(1):5-19.

[29] Irmieri A, Gibson W. Isolation and characterization of a noninfectious virionlike particle released from cells infected with human strains of cytomegalovirus. *Virology* 1983; 130:118-33.

[30] Schmolke S, Drescher P, Jahn G, Plachter B. Nuclear targeting of the tegument protein pp65 (UL83) of human cytomegalovirus: an unusual bipartite nuclear localization signal functions with other portions of the protein to mediate its efficient nuclear transport. *J Virol* 1995; 69:1071-8.

[31] Utz U, Britt W, Vugler L, Mach M. Identification of a neutralizing epitope on glycoprotein gp58 of human cytomegalovirus. *J Virol* 1989; 63:1995-2001.

[32] Frankenberg N, Pepperl-Klindworth S, Meyer RG, Plachter B. Identification of a conserved HLA-A2-restricted decapeptide from the IE1 protein (pUL123) of human cytomegalovirus. *Virology* 2002; 295(2):208-16.

[33] Wills MR, Carmichael AJ, Mynard K, Jin X, Weekes MP, Plachter B, et al. The human cytotoxic T-lymphocyte (CTL) response to cytomegalovirus is dominated by structural protein pp65: frequency, specificity, and T-cell receptor usage of pp65-specific CTL. *J Virol* 1996; 70:7569-79.

[34] Diamond DJ, York J, Sun JY, Wright CL, Forman SJ. Development of a candidate HLA A*0201 restricted peptide-based vaccine against human cytomegalovirus infection. *Blood* 1997; 90(5): 1751-67.

[35] Gallez-Hawkins G, Villacres MC, Li X, Sanborn MC, Lomeli NA, Zaia JA. Use of transgenic HLA A*0201/Kb and HHD II mice to evaluate frequency of cytomegalovirus IE1-derived peptide usage in eliciting human CD8 cytokine response. *J Virol* 2003; 77(7):4457-62.

[36] Pascolo S, Bervas N, Ure JM, Smith AG, Lemonnier FA, Perarnau B. HLA A2.1-restricted education and cytolytic activity of CD8(+) T lymphocytes from beta2 microglobulin (beta2m) HLA-A2.1 monochain transgenic H-2Db beta2m double knockout mice. *J Exp Med* 1997; 185(12):2043-51.

[37] Böhm V, Simon CO, Podlech J, Seckert CK, Gendig D, Deegen P, et al. The immune evasion paradox: immunoevasins of murine cytomegalovirus enhance priming of CD8 T cells by preventing negative feedback regulation. J Virol 2008; 82(23):11637-50.

5 [38] Pepperl-Klindworth S, Plachter B. Current perspectives in vaccine development. In: Reddehase MJ, editor. Cytomegaloviruses: Molecular Biology and Immunology. Caister Academic Press Ltd, Wymondham, Norfolk, U.K., 2006.

10 **[0081]** The features of the present invention disclosed in the specification, the claims, the sequence listing and/or the drawings may both separately and in any combination thereof be material for realizing the invention in various forms thereof.

SEQUENCE LISTING

15 **[0082]**

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20 <120> Viral particle released after infection of mammalian cells by human cytomegalovirus (HCMV) containing a fusion protein and use thereof

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<151> 2010-04-06

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 60
 Gly

Claims

1. A viral particle released after infection of mammalian cells by human cytomegalovirus (HCMV), wherein the viral particle is a dense body, wherein
 - a) the particle is surrounded by a lipid membrane in which viral glycoproteins are embedded,

- b) the particle contains neither viral DNA nor capsids; and
- c) the particle contains a fusion protein comprising one or more parts of the T-cell antigen pp65 and at least one antigenic heterologous peptide,

and wherein the at least one antigenic heterologous peptide is inserted at amino acid position W175 or A534 of the amino acid sequence of the T-cell antigen pp65 of SEQ ID NO: 1.

2. The viral particle according to claim 1, wherein the at least one antigenic heterologous peptide is inserted at amino acid position W175 of the amino acid sequence of the T-cell antigen pp65.
3. The viral particle according to any one of claims 1 to 2, wherein the amino acid sequence of the T-cell antigen pp65 comprises an amino acid sequence according to SEQ ID NO: 1.
4. The viral particle according to any one of claims 1 to 3, wherein the particle is highly antigenic.
5. The viral particle according to any one of claims 1 to 4, wherein the particle is capable of inducing the formation of neutralizing antibodies.
6. The viral particle according to any one of claims 1 to 5, wherein the particle is capable of inducing a CD8⁺ T lymphocyte response.
7. The viral particle according to any one of claims 1 to 6, wherein the at least one antigenic heterologous peptide is a MHC-class I presented antigen.
8. The viral particle according to any one of claims 1 to 7, wherein the at least one antigenic heterologous peptide comprises or is formed of one or more parts of one or more proteins which is/are different from pp65.
9. The viral particle according to any one of claims 1 to 8, wherein the at least one antigenic heterologous peptide comprises or is one or more parts of an HCMV glycoprotein.
10. The viral particle according to any one of claims 1 to 9, wherein the at least one antigenic heterologous peptide comprises or is one or more parts of the HCMV glycoprotein gB.
11. The viral particle according to any one of claims 1 to 9, wherein the at least one antigenic heterologous peptide comprises or is one or more parts of the HCMV glycoprotein gH.
12. The viral particle according to any one of claims 1 to 9, wherein the at least one antigenic heterologous peptide comprises or consists of at least two HCMV glycoproteins which are variants of a particular glycoprotein from different HCMV strains.
13. The viral particle according to claim 12, wherein one of the at least two variants of the particular glycoprotein is the variant of the HCMV Towne strain and the other of the at least two variants of the particular glycoprotein is the variant of the HCMV Ad169 strain.
14. The viral particle according to any one of claims 12 to 13, wherein the glycoprotein is the gB protein of HCMV.
15. The viral particle according to any one of claims 1 to 8, wherein the at least one antigenic heterologous peptide comprises or is one or more parts of the HCMV protein IE1.
16. The viral particle according to any one of claims 1 to 8, wherein the at least one antigenic heterologous peptide comprises or is one or more parts of an HCMV glycoprotein and one or more parts of the HCMV protein IE1.
17. The viral particle according to any one of claims 1 to 8, wherein the at least one antigenic heterologous peptide is one or more parts of a protein which is part of a human pathogen other than HCMV.
18. The viral particle according to claim 17, wherein the protein which is part of a human pathogen other than HCMV, is a protein against which cytotoxic T lymphocytes are formed in humans upon natural infection of humans with the human pathogen other than HCMV.

19. The viral particle according to claim 18, wherein the human pathogen other than HCMV is human pathogen selected from the group comprising HIV-1, HBV, HCV and influenza.
20. The viral particle according to any one of claims 1 to 19, wherein the fusion protein is a fusion protein comprising a full-length T-cell antigen pp65 and at least one antigenic heterologous peptide.
21. The viral particle according to any one of claims 1 to 20, or a plurality thereof, for the manufacture of a medicament for the treatment and/or prevention of a disease.
22. The viral particle according to any one of claims 1 to 20, or a plurality thereof, for use in a method for the treatment and/or prevention of a disease.
23. The viral particle according to any one of claims 21 to 22, wherein the disease is a disease which can be treated and/or prevented by the formation of neutralizing antibodies against the at least one antigenic heterologous peptide or a derivative thereof, or by the induction of a CD8⁺ T lymphocyte response against the at least one antigenic heterologous peptide or a derivative thereof.
24. The viral particle according to any one of claims 1 to 20, or a plurality thereof, for the manufacture of a vaccine.
25. The viral particle according to claim 24, wherein the vaccine is for the treatment and/or prevention of HCMV infection.
26. The viral particle according to claim 24, wherein the vaccine is for the treatment and/or prevention of side effects of transplantation.
27. The viral particle according to claim 26, wherein the transplantation is transplantation of a solid organ or haematopoietic stem cells.
28. The viral particle according to any one of claims 26 to 27, wherein the side effect is caused by or is going along with a HCMV infection.
29. Use of a viral particle according to any one of claims 1 to 20 for the manufacture of a medicament for the treatment and/or prevention of a diseases or side effects, whereby the disease or side effects is a disease or are side effects as defined in any of the preceding claims.
30. Use of a viral particle according to any one of claims 1 to 20 for the manufacture of a vaccine for the treatment and/or prevention of a disease or side effects, whereby the disease or side effects is a disease or are side effects as defined in any of the preceding claims.

Patentansprüche

1. Virales Partikel, das nach Infektion von Säugetierzellen durch menschliches Cytomegalovirus (HCMV) freigesetzt ist, wobei das virale Partikel ein "Dense Body" ist, wobei
 - a) das Partikel von einer Lipidmembran umgeben ist, in der virale Glykoproteine eingebettet sind,
 - b) das Partikel weder virale DNA noch Kapside enthält; und
 - c) das Partikel ein Fusionsprotein enthält, welches ein oder mehrere Teile des T-Zell-Antigens pp65 und wenigstens ein antigenes heterologes Peptid umfasst,und wobei das wenigstens eine antigene heterologe Peptid an Aminosäureposition W175 oder A534 der Aminosäuresequenz des T-Zell-Antigens pp65 gemäß SEQ.ID.NO: 1 eingefügt ist.
2. Virales Partikel nach Anspruch 1, wobei das wenigstens eine antigene heterologe Peptid an Aminosäureposition W175 der Aminosäuresequenz des T-Zell-Antigens pp65 eingefügt ist.
3. Virales Partikel nach einem der Ansprüche 1 bis 2, wobei die Aminosäuresequenz des T-Zell-Antigens pp65 eine Aminosäuresequenz nach SEQ.ID.NO: 1 umfasst.

4. Virales Partikel nach einem der Ansprüche 1 bis 3, wobei das Partikel hoch antigen ist.
5. Virales Partikel nach einem der Ansprüche 1 bis 4, wobei das Partikel in der Lage ist, die Bildung neutralisierender Antikörper zu induzieren.
- 5 6. Virales Partikel nach einem der Ansprüche 1 bis 5, wobei das Partikel in der Lage ist, eine CD8⁺-T-Lymphozytenantwort zu induzieren.
- 10 7. Virales Partikel nach einem der Ansprüche 1 bis 6, wobei das wenigstens eine antigene heterologe Peptid ein MHC-Klasse I-präsentiertes Antigen ist.
8. Virales Partikel nach einem der Ansprüche 1 bis 7, wobei das wenigstens eine antigene heterologe Peptid einen oder mehrere Teile von einem oder mehreren Proteinen, die verschieden sind von pp65, umfasst oder aus diesem/diesen ausgebildet ist.
- 15 9. Virales Partikel nach einem der Ansprüche 1 bis 8, wobei das wenigstens eine antigene heterologe Peptid einen oder mehrere Teile eines HCMV-Glykoproteins umfasst oder aus diesem/diesen besteht.
- 20 10. Virales Partikel nach einem der Ansprüche 1 bis 9, wobei das wenigstens eine antigene heterologe Peptid einen oder mehrere Teile des HCMV-Glykoproteins gB umfasst oder aus diesem/diesen besteht.
11. Virales Partikel nach einem der Ansprüche 1 bis 9, wobei das wenigstens eine antigene heterologe Peptid einen oder mehrere Teile des HCMV-Glykoproteins gH umfasst oder aus diesem/diesen besteht.
- 25 12. Virales Partikel nach einem der Ansprüche 1 bis 9, wobei das wenigstens eine antigene heterologe Peptid wenigstens zwei HCMV-Glykoproteine umfasst oder daraus besteht, welche Varianten eines speziellen Glykoproteins von verschiedenen HCMV-Stämmen sind.
- 30 13. Virales Partikel nach Anspruch 12, wobei eine der wenigstens zwei Varianten des speziellen Glykoproteins die Variante des Stammes HCMV Towne ist, und die andere der wenigstens zwei Varianten des speziellen Glykoproteins die Variante des Stammes HCMV Ad169 ist.
14. Virales Partikel nach einem der Ansprüche 12 bis 13, wobei das Glykoprotein das gB-Protein von HCMV ist.
- 35 15. Virales Partikel nach einem der Ansprüche 1 bis 8, wobei das wenigstens eine antigene heterologe Peptid einen oder mehrere Teile des HCMV-Proteins IE1 umfasst oder aus diesem/diesen besteht.
16. Virales Partikel nach einem der Ansprüche 1 bis 8, wobei das wenigstens eine antigene heterologe Peptid einen oder mehrere Teile eines HCMV-Glykoproteins und einen oder mehrere Teile des HCMV-Proteins IE1 umfasst oder aus diesen besteht.
- 40 17. Virales Partikel nach einem der Ansprüche 1 bis 8, wobei das wenigstens eine antigene heterologe Peptid ein oder mehrere Teile eines Proteins ist, welches Teil eines von HCMV verschiedenen menschlichen Pathogens ist.
- 45 18. Virales Partikel nach Anspruch 17, wobei das Protein, das Teil eines von HCMV verschiedenen humanen Pathogens ist, ein Protein ist, gegen das zytotoxische T-Lymphozyten in Menschen nach natürlicher Infektion von Menschen mit dem von HCMV verschiedenen humanen Pathogen gebildet werden.
- 50 19. Virales Partikel nach Anspruch 18, wobei das von HCMV verschiedene humane Pathogen ein humanes Pathogen ist, welches aus der Gruppe ausgewählt ist umfassend HIV-1, HBV, HCV und Influenza.
20. Virales Partikel nach einem der Ansprüche 1 bis 19, wobei das Fusionsprotein ein Fusionsprotein ist, das ein Volllängen-T-Zell-Antigen pp65 und wenigstens ein antigenes heterologes Peptid umfasst.
- 55 21. Virales Partikel nach einem der Ansprüche 1 bis 20, oder eine Vielzahl davon, für die Herstellung eines Medikamentes für die Behandlung und/oder Prävention einer Erkrankung.
22. Virales Partikel nach einem der Ansprüche 1 bis 20, oder eine Vielzahl davon, zur Verwendung in einem Verfahren

für die Behandlung und/oder Prävention einer Erkrankung.

23. Virales Partikel nach einem der Ansprüche 21 bis 22, wobei die Erkrankung eine Erkrankung ist, die behandelt und/oder verhindert werden kann durch die Bildung von neutralisierenden Antikörpern gegen das wenigstens eine antigene heterologe Peptid oder ein Derivat davon, oder durch die Induktion einer CD8⁺-T-Lymphozytenantwort gegen das wenigstens eine antigene heterologe Peptid oder ein Derivat davon.

24. Virales Partikel nach einem der Ansprüche 1 bis 20, oder eine Vielzahl davon, für die Herstellung eines Vakzins.

25. Virales Partikel nach Anspruch 24, wobei das Vakzin für die Behandlung und/oder Prävention von HCMV-Infektion ist.

26. Virales Partikel nach Anspruch 24, wobei das Vakzin für die Behandlung und/oder Prävention von Transplantationsnebenwirkungen ist.

27. Virales Partikel nach Anspruch 26, wobei die Transplantation Transplantation eines soliden Organs oder hämatopoetischer Stammzellen ist.

28. Virales Partikel nach einem der Ansprüche 26 bis 27, wobei die Nebenwirkung durch eine HCMV-Infektion verursacht ist oder mit dieser einhergeht.

29. Verwendung eines viralen Partikels nach einem der Ansprüche 1 bis 20 für die Herstellung eines Medikaments für die Behandlung und/oder Prävention einer Erkrankung oder von Nebenwirkungen, wobei die Erkrankung oder Nebenwirkungen eine Erkrankung oder Nebenwirkungen ist/sind, wie in einem der vorhergehenden Ansprüche definiert.

30. Verwendung eines viralen Partikels nach einem der Ansprüche 1 bis 20 für die Herstellung eines Vakzins für die Behandlung und/oder Prävention einer Erkrankung oder von Nebenwirkungen, wobei die Erkrankung oder Nebenwirkungen eine Erkrankung oder Nebenwirkungen ist/sind, wie in einem der vorhergehenden Ansprüche definiert.

Revendications

1. Particule virale libérée après infection de cellules de mammifères par le cytomégalo virus humain (CMVH), où la particule virale est un corps dense, où

- a) la particule est entourée par une membrane lipidique à laquelle des glycoprotéines virales sont intégrées,
- b) la particule ne contient ni ADN viral ni capsides ; et
- c) la particule contient une protéine de fusion comprenant une ou plusieurs parties de l'antigène pp65 de cellule T et au moins un peptide hétérologue antigène,

et où l'au moins un peptide hétérologue antigène est inséré en position d'acide aminé W175 ou A534 de la séquence d'acides aminés de l'antigène pp65 de cellule T de SEQ ID NO: 1.

2. Particule virale selon la revendication 1, où l'au moins un peptide hétérologue antigène est inséré en position d'acide aminé W175 de la séquence d'acides aminés de l'antigène pp65 de cellule T.

3. Particule virale selon l'une quelconque des revendications 1 à 2, où la séquence d'acides aminés de l'antigène pp65 de cellule T comprend une séquence d'acides aminés selon SEQ ID NO: 1.

4. Particule virale selon l'une quelconque des revendications 1 à 3, où la particule est fortement antigène.

5. Particule virale selon l'une quelconque des revendications 1 à 4, où la particule est capable d'induire la formation d'anticorps neutralisants.

6. Particule virale selon l'une quelconque des revendications 1 à 5, où la particule est capable d'induire une réponse à lymphocytes T CD8⁺.

7. Particule virale selon l'une quelconque des revendications 1 à 6, où l'au moins un peptide hétérologue antigène est

un antigène présenté par le CMH de classe I.

- 5 8. Particule virale selon l'une quelconque des revendications 1 à 7, où l'au moins un peptide hétérologue antigène comprend ou est formé d'une ou de plusieurs parties d'une ou de plusieurs protéines qui est/sont différente(s) de pp65.
9. Particule virale selon l'une quelconque des revendications 1 à 8, où l'au moins un peptide hétérologue antigène comprend ou est une ou plusieurs parties d'une glycoprotéine du CMVH.
- 10 10. Particule virale selon l'une quelconque des revendications 1 à 9, où l'au moins un peptide hétérologue antigène comprend ou est une ou plusieurs parties de la glycoprotéine gB du CMVH.
11. Particule virale selon l'une quelconque des revendications 1 à 9, où l'au moins un peptide hétérologue antigène comprend ou est une ou plusieurs parties de la glycoprotéine gH du CMVH.
- 15 12. Particule virale selon l'une quelconque des revendications 1 à 9, où l'au moins un peptide hétérologue antigène comprend ou est constitué d'au moins deux glycoprotéines du CMVH qui sont des variantes d'une glycoprotéine particulière de souches du CMVH différentes.
- 20 13. Particule virale selon la revendication 12, où l'une des au moins deux variantes de la glycoprotéine particulière est la variante de la souche Towne du CMVH et l'autre des au moins deux variantes de la glycoprotéine particulière est la variante de la souche Ad169 du CMVH.
- 25 14. Particule virale selon l'une quelconque des revendications 12 à 13, où la glycoprotéine est la protéine gB du CMVH.
15. Particule virale selon l'une quelconque des revendications 1 à 8, où l'au moins un peptide hétérologue antigène comprend ou est une ou plusieurs parties de la protéine IE1 du CMVH.
- 30 16. Particule virale selon l'une quelconque des revendications 1 à 8, où l'au moins un peptide hétérologue antigène comprend ou est une ou plusieurs parties d'une glycoprotéine du CMVH et une ou plusieurs parties de la protéine IE1 du CMVH.
- 35 17. Particule virale selon l'une quelconque des revendications 1 à 8, où l'au moins un peptide hétérologue antigène est une ou plusieurs parties d'une protéine qui fait partie d'un pathogène humain différent du CMVH.
18. Particule virale selon la revendication 17, où la protéine qui fait partie d'un pathogène humain différent du CMVH est une protéine contre laquelle les lymphocytes T cytotoxiques sont formés chez l'humain après infection naturelle d'humains par le pathogène humain différent du CMVH.
- 40 19. Particule virale selon la revendication 18, où le pathogène humain différent du CMVH est le pathogène humain choisi dans le groupe comprenant VIH-1, VHB, VHC et l'influenza.
20. Particule virale selon l'une quelconque des revendications 1 à 19, où la protéine de fusion est une protéine de fusion comprenant un antigène pp65 de cellule T de longueur totale et au moins un peptide hétérologue antigène.
- 45 21. Particule virale selon l'une quelconque des revendications 1 à 20, ou multitude de celles-ci, pour la fabrication d'un médicament destiné au traitement prophylactique et/ou thérapeutique d'une maladie.
- 50 22. Particule virale selon l'une quelconque des revendications 1 à 20, ou multitude de celles-ci, pour leur utilisation dans une méthode de traitement prophylactique et/ou thérapeutique d'une maladie.
23. Particule virale selon l'une quelconque des revendications 21 à 22, où la maladie est une maladie qui peut être traitée et/ou prévenue par la formation d'anticorps neutralisant dirigés contre au moins un peptide hétérologue antigène ou l'un de ses dérivés, ou par l'induction d'une réponse à lymphocytes T CD8⁺ contre au moins un peptide hétérologue antigène ou l'un de ses dérivés.
- 55 24. Particule virale selon l'une quelconque des revendications 1 à 20, ou multitude de celles-ci, pour la fabrication d'un vaccin.

25. Particule virale selon la revendication 24, où le vaccin est destiné au traitement prophylactique et/ou thérapeutique d'une infection par le CMVH.

26. Particule virale selon la revendication 24, où le vaccin est destiné au traitement prophylactique et/ou thérapeutique des effets secondaires de la transplantation.

27. Particule virale selon la revendication 26, où la transplantation et une transplantation d'un organe solide ou de cellules souches hématopoïétiques.

28. Particule virale selon l'une quelconque des revendications 26 à 27, où l'effet secondaire est provoqué par ou accompagne une infection par le CMVH.

29. Utilisation d'une particule virale selon l'une quelconque des revendications 1 à 20 pour la fabrication d'un médicament destiné au traitement prophylactique et/ou thérapeutique d'une maladie ou d'effets secondaires, où la maladie ou les effets secondaires sont une maladie ou des effets secondaires comme défini dans l'une quelconque des revendications précédentes.

30. Utilisation d'une particule virale selon l'une quelconque des revendications 1 à 20 pour la fabrication d'un vaccin destiné au traitement prophylactique et/ou thérapeutique d'une maladie ou d'effets secondaires, où la maladie ou les effets secondaires sont une maladie ou des effets secondaires comme défini dans l'une quelconque des revendications précédentes.

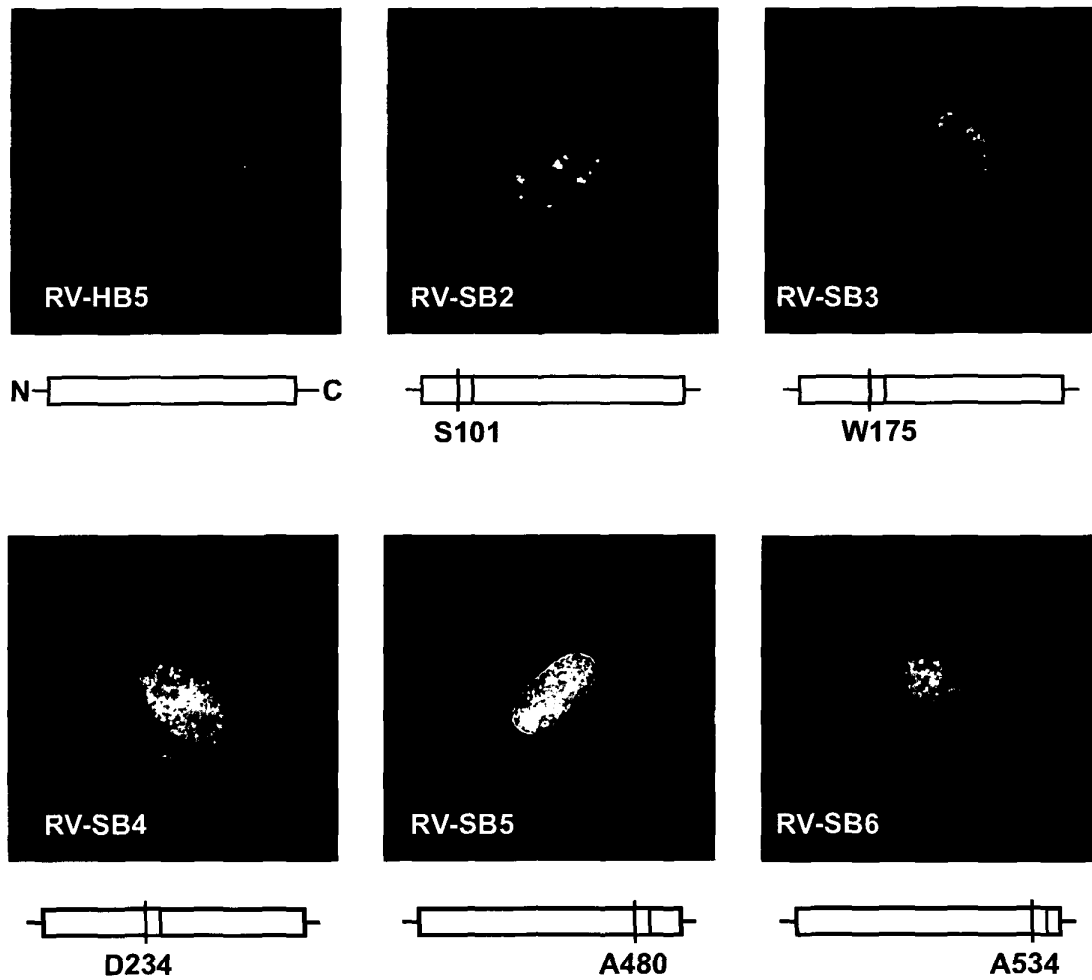


FIG. 1

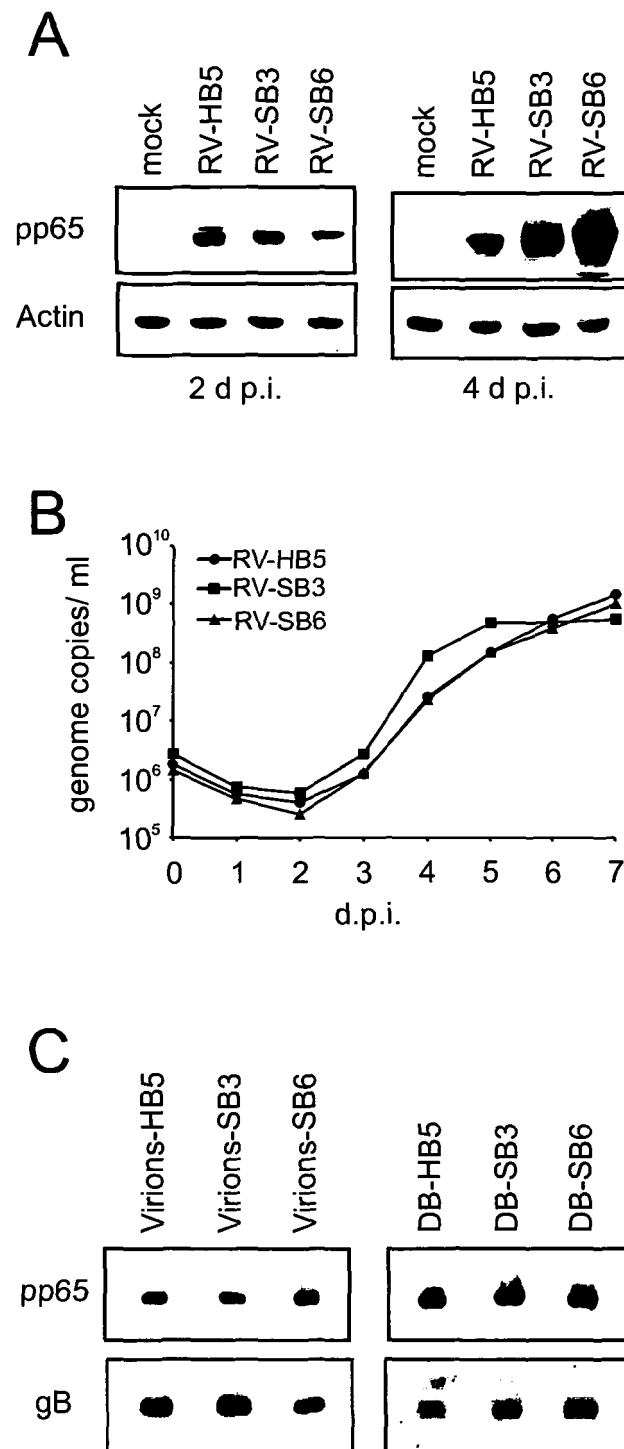


FIG. 2

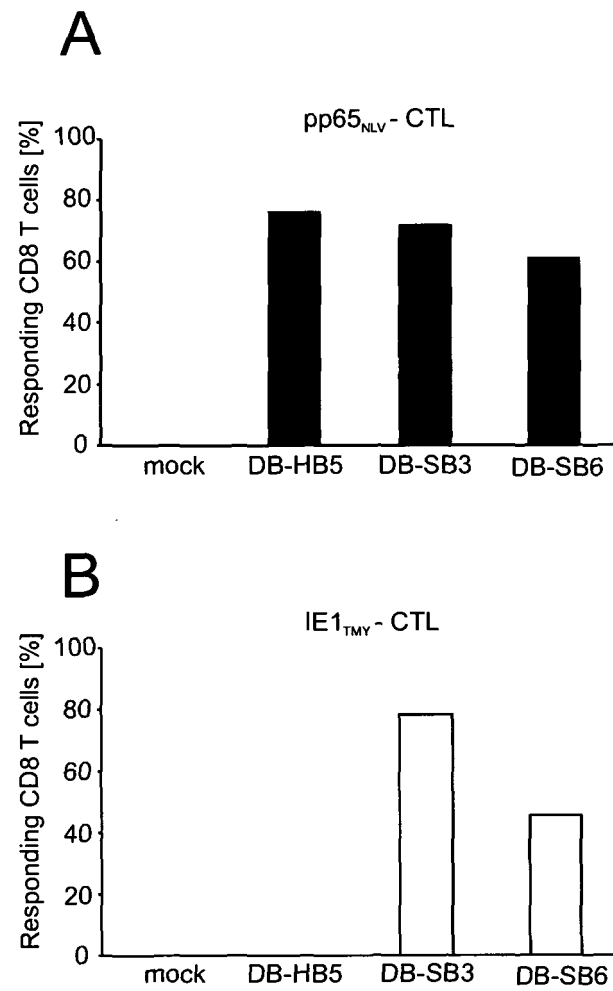


FIG. 3

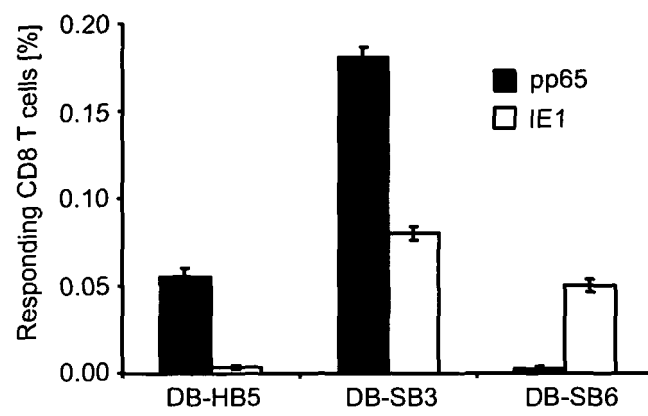


FIG. 4

mesrgrrcpe	misvlgpisg	hvlkavfsrg	dtpvlphe	llqtfihvrv	sqpslilvsq
ytpdstpchr	gdnqlqvqht	yftgsevenv	svnvhnptgr	sicpsqepms	iyvyalplkm
lnipsinvhh	ypsaaerkhr	hlpvadavih	asgkqmwqar	ltvsglawtr	qqnqwkepdv
yytsafvfpt	kdvalrhvvc	ahelvcsmen	tratkmqvig	dgyvkvytes	fcedvpsgkl
fmhvltlgsdv	eedltmtrnp	qpfmrphern	gftvlpcknm	iikpgkishi	mldvaftshe
hfgllcpksi	pglsgisgnll	mngqqiflev	qairetvelr	qydpvaalff	fdidlllqrg
pqysehptft	sqyriqgkle	yrhtwdrhde	gaaqgdddvw	tsgsdsdeel	vtterktprv
tgggamagas	tsagrkrksa	ssatactagv	mtrgrlkaes	tvapeedtde	dsdneihnpa
vftwppwqag	ilarnlvpmv	atvqggqlky	qeffwdandi	yrifaelegv	wqpaaqpkrr
rhrqdalpgp	ciastpkkhr	g			

Fig. 5

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- WO 0053729 A [0006]
- EP 10003712 A [0082]

Non-patent literature cited in the description

- **PANDE, H ; LEE, T.D. ; CHURCHILL, M.A. ; ZAIA, J.A.** Structural analysis of a 64-kDa major structural protein of human cytomegalovirus (Towne): identification of a phosphorylation site and comparison to pp65 of HCMV (AD169). *Virology*, 1990, vol. 178 (1), 6-14 [0043]
- **MWINTSHIK ; BRENNAN DC.** Prevention and management of cytomegalovirus infection in solid-organ transplantation. *Expert Rev Anti Infect Ther*, 2007, vol. 5 (2), 295-304 [0080]
- **RUBIN RH.** The pathogenesis and clinical management of cytomegalovirus infection in the organ transplant recipient: the end of the 'silo hypothesis. *Curr Opin Infect Dis*, 2007, vol. 20 (4), 399-407 [0080]
- **ARTHURS SK ; EID AJ ; PEDERSEN RA ; DIERKHISING RA ; KREMERS WK ; PATEL R et al.** Delayed-onset primary cytomegalovirus disease after liver transplantation. *Liver Transpl*, 2007, vol. 13 (12), 1703-9 [0080]
- **ARTHURS SK ; EID AJ ; PEDERSEN RA ; KREMERS WK ; COSIO FG ; PATEL R et al.** Delayed-onset primary cytomegalovirus disease and the risk of allograft failure and mortality after kidney transplantation. *Clin Infect Dis*, 2008, vol. 46 (6), 840-6 [0080]
- **PEGGS KS.** Cytomegalovirus following stem cell transplantation: from pharmacologic to immunologic therapy. *Expert Rev Anti Infect Ther*, 2004, vol. 2 (4), 559-73 [0080]
- **HEBART H ; EINSELE H.** Clinical aspects of CMV infection after stem cell transplantation. *Hum Immunol*, 2004, vol. 65 (5), 432-6 [0080]
- **LJUNGMAN P.** Risk assessment in haematopoietic stem cell transplantation: viral status. *Best Pract Res Clin Haematol*, 2007, vol. 20 (2), 209-17 [0080]
- **BOECKH M ; NICHOLS WG ; PAPANICOLAOU G ; RUBIN R ; WINGARD JR ; ZAIA J.** Cytomegalovirus in hematopoietic stem cell transplant recipients: Current status, known challenges, and future strategies. *Biol Blood Marrow Transplant*, 2003, vol. 9 (9), 543-58 [0080]
- **BOECKH M ; NICHOLS WG.** The impact of cytomegalovirus serostatus of donor and recipient before hematopoietic stem cell transplantation in the era of antiviral prophylaxis and preemptive therapy. *Blood*, 2004, vol. 103 (6), 2003-8 [0080]
- Cytomegalovirus vaccines. **PLOTKIN SA.** Vaccines. Elsevier, 2008, 1147-54 [0080]
- **CHEERAN MC ; LOKENSGARD JR ; SCHLEISS MR.** Neuropathogenesis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. *Clin Microbiol Rev*, 2009, vol. 22 (1), 99-126 [0080]
- Vaccines for the 21st Century. **STRATTON KR ; DURCH JS ; LAWRENCE R.S.** A Tool for Decisionmaking. National Academy Press, 2001 [0080]
- **REDDEHASE MJ ; MUTTER W ; MÜNCH K ; BÜHRING HJ ; KOSZINOWSKI UH.** CD8-positive T lymphocytes specific for murine cytomegalovirus immediate-early antigens mediate protective immunity. *J Virol*, 1987, vol. 61, 3102-8 [0080]
- Adaptive Cellular Immunity to Human Cytomegalovirus. **WILLS MR ; CARMICHAEL AJ ; SISSONS JG.** Cytomegalovirus: Molecular Biology and Immunology. Caister Academic Press, 2006, 341-65 [0080]
- **WALTER EA ; GREENBERG PD ; GILBERT MJ ; FINCH RJ ; WATANABE KS ; THOMAS ED et al.** Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. *N Engl J Med*, 1995, vol. 333 (16), 1038-44 [0080]
- **HERR W ; PLACHTER B.** Cytomegalovirus and varicella-zoster virus vaccines in hematopoietic stem cell transplantation. *Expert Rev Vaccines*, 2009, vol. 8 (8), 999-1021 [0080]
- **SCHLEISS MR.** Cytomegalovirus vaccine development. *Curr Top Microbiol Immunol*, 2008, vol. 325, 361-82 [0080]
- **PASS RF ; ZHANG C ; EVANS A ; SIMPSON T ; ANDREWS W ; HUANG ML et al.** Vaccine prevention of maternal cytomegalovirus infection. *N Engl J Med*, 2009, vol. 360 (12), 1191-9 [0080]

- **BERNSTEIN DI ; REAP EA ; KATEN K ; WATSON A ; SMITH K ; NORBERG P et al.** Randomized, double-blind, Phase 1 trial of an alphavirus replicon vaccine for cytomegalovirus in CMV seronegative adult volunteers. *Vaccine*, 2009, vol. 28 (2), 484-93 [0080]
- **PEPPERL S ; MÜNSTER J ; MACH M ; HARRIS JR ; PLACHTER B.** Dense bodies of human cytomegalovirus induce both humoral and cellular immune responses in the absence of viral gene expression. *J Virol*, 2000, vol. 74 (13), 6132-46 [0080]
- **MERSSEMAN V ; BESOLD K ; REDDEHASE MJ ; WOLFRUM U ; STRAND D ; PLACHTER B et al.** Exogenous introduction of an immunodominant peptide from the nonstructural IE1 protein of human cytomegalovirus into the MHC class I presentation pathway by recombinant dense bodies. *J Gen Virol*, 2008, vol. 89, 369-79 [0080]
- **MERSSEMAN V ; BOHM V ; HOLTAPPELS R ; DEEGEN P ; WOLFRUM U ; PLACHTER B et al.** Refinement of strategies for the development of a human cytomegalovirus dense body vaccine. *Med Microbiol Immunol*, 2008, vol. 197 (2), 97-107 [0080]
- **BESOLD K ; FRANKENBERG N ; PEPPERL-KLINDWORTH S ; KUBALL J ; THEOBALD M ; HAHN G et al.** Processing and MHC class I presentation of human cytomegalovirus pp65-derived peptides persist despite gpUS2-11-mediated immune evasion. *J Gen Virol*, 2007, vol. 88, 1429-39 [0080]
- **TOWNSEND A ; OHLEN C ; BASTIN J ; LJUNG-GREN HG ; FOSTER L ; KARRE K.** Association of class I major histocompatibility heavy and light chains induced by viral peptides. *Nature*, 1989, vol. 340 (6233), 443-8 [0080]
- **BORST EM ; HAHN G ; KOSZINOWSKI UH ; MESSERLE M.** Cloning of the human cytomegalovirus (HCMV) genome as an infectious bacterial artificial chromosome in *Escherichia coli*: a new approach for construction of HCMV mutants. *J Virol*, 1999, vol. 73 (10), 8320-9 [0080]
- **WARMING S ; COSTANTINO N ; COURT DL ; JENKINS NA ; COPELAND NG.** Simple and highly efficient BAC recombineering using galK selection. *Nucleic Acids Res*, 2005, vol. 33 (4), 36 [0080]
- **HOBOM U ; BRUNE W ; MESSERLE M ; HAHN G ; KOSZINOWSKI UH.** Fast screening procedures for random transposon libraries of cloned herpesvirus genomes: mutational analysis of human cytomegalovirus envelope glycoprotein genes. *J Virol*, 2000, vol. 74 (17), 7720-9 [0080]
- **BESOLD K ; WILLS M ; PLACHTER B.** Immune evasion proteins gpUS2 and gpUS11 of human cytomegalovirus incompletely protect infected cells from CD8 T cell recognition. *Virology*, 2009, vol. 391 (1), 5-19 [0080]
- **IRMIERE A ; GIBSON W.** Isolation and characterization of a noninfectious virionlike particle released from cells infected with human strains of cytomegalovirus. *Virology*, 1983, vol. 130, 118-33 [0080]
- **SCHMOLKE S ; DRESCHER P ; JAHN G ; PLACHTER B.** Nuclear targeting of the tegument protein pp65 (UL83) of human cytomegalovirus: an unusual bipartite nuclear localization signal functions with other portions of the protein to mediate its efficient nuclear transport. *J Virol*, 1995, vol. 69, 1071-8 [0080]
- **UTZ U ; BRITT W ; VUGLER L ; MACH M.** Identification of a neutralizing epitope on glycoprotein gp58 of human cytomegalovirus. *J Virol*, 1989, vol. 63, 1995-2001 [0080]
- **FRANKENBERG N ; PEPPERL-KLINDWORTH S ; MEYER RG ; PLACHTER B.** Identification of a conserved HLA-A2-restricted decapeptide from the IE1 protein (pUL123) of human cytomegalovirus. *Virology*, 2002, vol. 295 (2), 208-16 [0080]
- **WILLS MR ; CARMICHAEL AJ ; MYNARD K ; JIN X ; WEEKES MP ; PLACHTER B et al.** The human cytotoxic T-lymphocyte (CTL) response to cytomegalovirus is dominated by structural protein pp65: frequency, specificity, and T-cell receptor usage of pp65-specific CTL. *J Virol*, 1996, vol. 70, 7569-79 [0080]
- **DIAMOND DJ ; YORK J ; SUN JY ; WRIGHT CL ; FORMAN SJ.** Development of a candidate HLA A*0201 restricted peptide-based vaccine against human cytomegalovirus infection. *Blood*, 1997, vol. 90 (5), 1751-67 [0080]
- **GALLEZ-HAWKINS G ; VILLACRES MC ; LI X ; SANBORN MC ; LOMELI NA ; ZAIA JA.** Use of transgenic HLA A*0201/Kb and HHD II mice to evaluate frequency of cytomegalovirus IE1-derived peptide usage in eliciting human CD8 cytokine response. *J Virol*, 2003, vol. 77 (7), 4457-62 [0080]
- **PASCOLO S ; BERVAS N ; URE JM ; SMITH AG ; LEMONNIER FA ; PERARNAU B.** HLA A2.1-restricted education and cytolytic activity of CD8(+) T lymphocytes from beta2 microglobulin (beta2m) HLA-A2.1 monochain transgenic H-2Db beta2m double knockout mice. *J Exp Med*, 1997, vol. 185 (12), 2043-51 [0080]
- **BÖHM V ; SIMON CO ; PODLECH J ; SECKERT CK ; GENDIG D ; DEEGEN P et al.** The immune evasion paradox: immunoevasins of murine cytomegalovirus enhance priming of CD8 T cells by preventing negative feedback regulation. *J Virol*, 2008, vol. 82 (23), 11637-50 [0080]
- **Current perspectives in vaccine development. PEPPERL-KLINDWORTH S ; PLACHTER B.** Cytomegaloviruses: Molecular Biology and Immunology. Academic Press Ltd, 2006 [0080]